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Reduced hepatic protein synthesis after long term ethanol treatment in fasted rats. Dependence on animal handling before measurement*

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Chronic ethanol intake has resulted in reduced *in vivo* incorporation of labelled amino acids into total liver protein [1–7], but also no changes [8–11] and increases [12] have been found. These incorporation studies suffer from incomplete control of the specific radioactivity of the precursor amino acid during the incorporation period. The rate of incorporation of any amino acid is a function of its specific radioactivity, and it has therefore been difficult to ascertain whether chronic ethanol intake really leads to a change in the rate of hepatic protein synthesis. In the present investigation we employed continuous infusion of the precursor amino acid (^{3}H-valine) and could thereby estimate the specific radioactivity of valine during the experiment, and measure the rate of protein synthesis.

Male Wistar rats from Møllegaard Hansens Avlslaboratorier, Ejby, Denmark, were divided into 16 weight-matched pairs (202 g mean body wt). They were pair-fed the experimental diets consisting of both liquid and solid food and otherwise treated as described previously [13, 14] (with the following dietary additions per kg solid food: 11.4 mg $ZnSO_4 \times 7\ H_2O$, 32.2 mg $CuSO_4 \times 5\ H_2O$, 295 mg $MnCl_2$, 5 g cystine and 7 g NaCl) for 58–65 days. In the present study ethanol constituted 25–30 per cent of the total energy consumed, i.e. approximately 10 g ethanol/kg rat/day given as a 16 per cent (v/v) solution ethanol *ad lib.*, while lipid (10 per cent w/v) replaced ethanol isoenergetically in the solution given to the control group. A preliminary study has shown no difference in ^{3}H-valine incorporation into liver protein between control rats pair-fed isoenergetic amounts of either lipid or sucrose. The lipid control diet was then chosen as control on the basis of results from a previous study [14]. The liquid was replaced by water 24 hr before sacrifice, and all rats were fasted for the last 16 hr of this period. Ethanol-fed and control rats were each divided into 3 subgroups; one received no further treatment, another was injected i.p. with 4 mg dexamethasone/kg rat, while the third group obtained a similar volume (1 ml/kg rat) of sterile 0.9 per cent saline 60 min before *in vivo* measurement of protein synthesis started. About 55 min later the rats were ether anaesthetized, the abdominal cavity opened, and a cannula was inserted into a side branch of vena cava inferior. A solution containing 50 μCi [2,3 – ^{3}H] valine (TRK 533 36 Ci/mmol, Radiochemical Centre, Amersham, U.K.), 15 μmoles valine and 50 I.U. heparin per ml 0.9 per cent saline, was infused for 30 min at a rate of 194 μl/min during 8 min and 39 μl/min during the following 22 min. After ligation, smaller liver samples were excised at 11 and 20 min, and the rest of the liver was taken after 30 min. All samples were briefly rinsed in ice-cold saline and freeze-clamped in liquid nitrogen. Blood was collected from the abdominal aorta at the end of infusion, and plasma was separated. Liver protein was determined according to Lowry [15], liver DNA according to Burton [16], and liver RNA was measured by the orcinol reagent [17]. Plasma corticosterone was determined by a competitive protein-binding radioassay [18] using [1, 2, 6, 7 (*n*) – ^{3}H] corticosterone (TRK 406 80,000–100,000 mCi/mmol, Radiochemical Centre, Amersham, U.K.). Incorporation of ^{3}H-valine into protein, acid-soluble ^{3}H-valine as well as total acid-soluble valine in both liver and plasma were determined as detailed before [4, 5]. By amino acid analysis it was checked that all acid-soluble ^{3}H-radioactivity in livers was eluted quantitatively in the valine peak as did pure valine. No radioactivity was found in any other peak. This was found in both ethanol-fed and control rats, indicating that the total acid-soluble radioactivity represented unmetabolized, free valine in both groups. The rate of protein synthesis was calculated for two time intervals, 11–20 min and 20–30 min, by dividing the increase in protein specific radioactivity during the interval with the mean specific activity of free, intracellular valine in the interval and expressed as the mean for the two intervals. The specific precursor radioactivity changed markedly during the interval 0–11 min, and this period was therefore omitted. The free intracellular valine was calculated per ml of the intracellular water assuming that the extracellular phase constituted 30 per cent and that intracellular water constituted 47 per cent of liver wet weight [19]. Wilcoxon's test (complete randomization) was used to determine statistical significance, $\alpha < 0.05$ was considered as significant.

There was no mortality in any group during the feeding period, while one rat died during anaesthesia. Daily ethanol intake averaged 9.2 g/kg rat/day. Mean daily weight gain was 2.9 g in the control group and 2.8 g in the ethanol group. There was no significant difference between ethanol-fed groups and corresponding control groups in final body weight (Table 1). The relative liver weight as well as the relative hepatic protein content was higher in ethanol-treated rats, while no difference was found between pair-fed groups in liver DNA and RNA contents (mg/100 g body weight).

Our experiments demonstrated that the incorporation of ^{3}H-valine into protein and the calculated rate of protein synthesis was reduced in chronic ethanol-fed rats that received no acute treatment (Table 2). A reduction in ^{3}H-valine incorporation was still seen in ethanol-fed rats that had received saline or dexamethasone i.p. acutely when compared to controls given the same treatment. These latter differences were, however, not present with respect to rates of protein synthesis. Saline i.p. increased the protein synthetic rate somewhat in ethanol-fed rats, while dexamethasone i.p. increased the rate in ethanol-fed rats both compared to the 'no-handle' ethanol group and to the saline-injected ethanol group. This increase which did not occur in the control rats, completely abolished any difference between ethanol-fed and control rats. Studies of incorporation into plasma proteins (Table 2) were performed only to see whether ethanol treatment increased the leakage of newly synthesized proteins into the blood. This was not the case. These results indicate that the 'stress' of being injected was sufficient to influence the outcome of an experiment of the present type considerably. Catecholamines or some endogenous steroid hormone could perhaps have been the mediator of this effect, since dexa-

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Table 1. Effect of ethanol feeding on body weight, liver wet weight and liver content of DNA, RNA and protein*

Treatment		Number of rats	Final body weight (g)	Liver			
Long term	Acute			Wet wt (g/100 g body wt)	DNA (mg/100 g body wt)	RNA (mg/100 g body wt)	Protein (mg/100 g body wt)
Ethanol	No	6	385 ± 5	2.88 ± 0.07†	10.2 ± 0.3	29.9 ± 0.6	698 ± 6†
	Saline i.p.	4	375 ± 7	2.94 ± 0.10†	10.0 ± 0.4	29.0 ± 0.9	708 ± 32†
	Dexamethasone i.p.	5	370 ± 13	2.97 ± 0.08†	10.4 ± 0.2	29.3 ± 0.3	693 ± 35†
Control	No	6	378 ± 17	2.68 ± 0.05	9.84 ± 0.34	29.7 ± 0.7	641 ± 9
	Saline i.p.	4	396 ± 10	2.54 ± 0.10	9.64 ± 0.28	27.8 ± 1.5	589 ± 30
	Dexamethasone i.p.	6	379 ± 10	2.59 ± 0.03	9.71 ± 0.21	27.9 ± 0.2	605 ± 8

* Rats were pair-fed either ethanol or control diets for 58–65 days. The acute treatments were given 90 min prior to sacrifice. Mean values ± SEM are given.

† $\alpha<0.05$ with respect to the corresponding control group according to Wilcoxon's complete randomization test.

methasone had a more pronounced extinguishing effect. The level of the principle adrenal steroid hormone in the rat, corticosterone, was the same in all groups at the end of the infusion (data not shown). Our results emphasize the importance of detailed description of procedures connected to the measurements of protein synthesis.

The intracellular specific radioactivity of precursor (Fig. 1A) was somewhat lower in ethanol-fed rats at all points of time. The concentration of ^{3}H-valine was not lower after ethanol treatment (Fig. 1B). Due to a higher concentration of intracellular valine in ethanol-treated rats, the specific radioactivity was lower. This lack of correspondence showed that even by infusing valine at high concentration, direct measurement of specific radioactivities was required as a basis for calculation of protein synthetic rates. It should be noted that the effect of long term ethanol consumption on protein synthesis would have been relatively greater than reported (Table 2) if extracellular specific radioactivity had been used as a basis of calculation, since this value was higher in ethanol-treated rats than in controls (Fig. 1A, right panel).

Dexamethasone, increasing the rate of protein synthesis in the ethanol-fed rats to control level, might lead to increased level of some amino acid critical to the rate of protein synthesis. Glycine, which is not regulating the rate of protein synthesis, was the only hepatic amino acid that increased after dexamethasone treatment (data not shown). This indicated that dexamethasone influenced protein synthesis by a mechanism independent of amino acid supply. There was no difference between untreated ethanol and control rats with respect to the level of glycine. Furthermore, there was no substantial reduction in the hepatic concentration of the amino acids studied (aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, histidine and lysine) after ethanol consumption. In addition to the increased intracellular levels of valine registered, we also found increased intracellular levels of leucine and isoleucine. Increased hepatic leucine concentrations have also been reported in other studies [3, 12, 20]. These observations may indicate an increased rate of protein degradation in the liver. In another investigation, using fed rats, we were not able to observe any increase in branched amino acid levels [15].

Chronic ethanol-fed rats had reduced hepatic protein synthesis as measured by using continuous infusion of ^{3}H-valine. However, acute treatment (i.p. injection) one

Table 2. Effect of long term ethanol feeding and acute treatment on hepatic protein synthesis and ^{3}H-valine incorporation into plasma proteins*

Treatment		Incorporation into liver protein (dpm/mg protein/min)	Liver protein synthesis (nmoles valine/ 100 g body wt/min)	Incorporation into plasma protein (dpm/mg protein)
Long term	Acute			
Ethanol	No	101 ± 10† (6)	54.4 ± 4.3† (6)	226 ± 25† (5)
Control	No	226 ± 23 (6)	86.0 ± 5.9 (6)	395 ± 73 (6)
Ethanol	Saline i.p.	142 ± 22† (4)	69.9 ± 6.1 (4)	286 ± 41 (3)
Control	Saline i.p.	217 ± 11 (4)	78.1 ± 22.5 (4)	411 ± 59 (4)
Ethanol	Dexamethasone i.p.	180 ± 18†‡ (5)	94.5 ± 6.8§ (5)	246 ± 41† (5)
Control	Dexamethasone i.p.	261 ± 10 (6)	82.4 ± 3.3 (6)	596 ± 30 (6)

* Rats were pair-fed either ethanol or control diets for 58–65 days. The acute treatment was given 60 min prior to the measurement of hepatic protein synthesis, which was determined from a 30 min continuous infusion of 122 μCi ^{3}H-valine, specific activity 3.33 Ci/mol. Values were calculated for the period 11–30 min of infusion for liver protein incorporation and synthesis, while incorporation into plasma proteins was determined at the end of infusion. Mean values ± SEM are given with the number of rats in parenthesis.

† $\alpha<0.05$, with respect to pair-fed control group.

‡ $\alpha<0.05$, with respect to the ethanol group without acute treatment.

§ $\alpha<0.05$, with respect to both other ethanol groups.

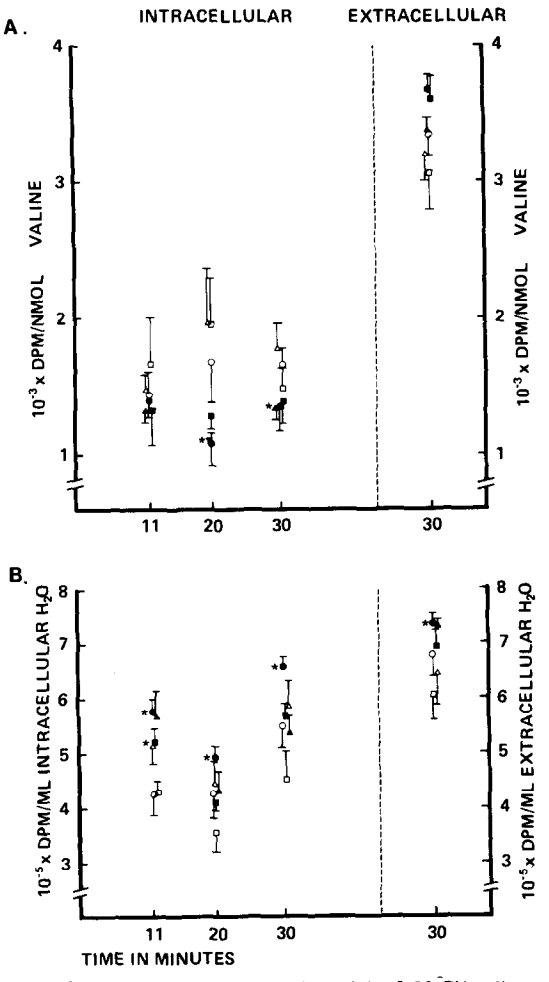

Fig. 1. ^{3}H-valine (122 μCi, specific activity 3.33 Ci/mol) was infused intravenously in rats throughout a 30 min period and liver samples were taken at 11, 20 and 30 min. Plasma was collected at 30 min. The rats were pair-fed either ethanol or control diet for 58–65 days and fasted for approximately 16 hr previous to the infusion. In addition, 60 min before the infusion started, the rats received either an i.p. injection of saline or dexamethasone, or no treatment at all. All values represent means of the number of animals detailed in Table 1. *α<0.05, ethanol group significantly different from pair-fed control group. (A) Specific radioactivity of valine in liver and plasma. (B) ^{3}H-valine radioactivity in liver and plasma. ● Ethanol; ■ ethanol + saline; ▲ ethanol + dexamethasone; ○ control; □ control + saline; △ control + dexamethasone.

hour before measurement influenced the protein synthetic rate considerably. The intrahepatic protein content was elevated in the ethanol-fed rats. These apparent conflicting results indicated either that protein synthesis measured in short term labelling experiments did not represent synthesis of total liver protein, or that reduced synthesis was compensated by changes in protein degradation or transport. More studies are needed to clarify the mechanism(s).

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